PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 06 November 2000 (06.11.00)	
International application No. PCT/US00/05820	**Applicant's or agent's file reference** 05213-0463WP
International filing date (day/month/year) 03 March 2000 (03.03.00)	**Priority date** (day/month/year) 04 March 1999 (04.03.99)
Applicant NARUM, David, L. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

29 September 2000 (29.09.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Claudio Borton
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Articl 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
05213-0463WP		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 00/ 05820	03/03/2000	04/03/1999
Applicant		
ENTREMED, INC. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [X] furnished subsequently to this Authority in written form.

   [X] furnished subsequently to this Authority in computer readble form.

   [X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 3

   [X] as suggested by the applicant.

   [ ] None of the figures.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

INTERNATIONAL SEARCH REPORT

tional application No.
PCT/US 00/05820

Box I Observations wher certain claims were f und unsearchable (Continuation of item 1 f first sh et)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 8 (partially), 10 and 13 to 16 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark n Pr test

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No
PC[redacted]S 00/05820

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K16/20 C12N5/20 A61K39/395 A61P33/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, EPO-Internal, WPI Data, PAJ, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 40766 A (US HEALTH) 19 December 1996 (1996-12-19) abstract page 1, line 11-31 page 2, line 2 page 2, line 32 -page 3, line 3 page 6, line 20 - line 36 page 5, line 1 - line 5 page 7, line 17 - line 19 page 7, line 20 -page 8, line 5 page 9, line 10 - line 19 page 15, line 31 - line 35 figure 1 --- -/--	1-4,6-16

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 August 2000	24.08.00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Montrone, M

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 07353 A (US HEALTH) 16 March 1995 (1995-03-16) abstract page 5, line 12 - line 22 page 6, line 22 -page 7, line 17 page 9, line 25 page 11, line 7 - line 37 page 12, line 10 -page 13, line 12 page 25, line 4 - line 12 page 27, line 30 -page 28, line 10	1-4,6-16
X	B. KIM LEE SIM: "Delineation of functional regions on Plasmodium falciparum EBA-175 by antibodies eluded from immune complexes" MOL. BIOCHEM. PARASITOLOGY, vol. 95, 1998, pages 183-192, XP000915150 abstract page 184; table 1 page 186, column 2, paragraph 5 page 187; figure 1 page 188, column 1, paragraph 4 page 189, column 1, paragraph 3 -column 2, paragraph 1 page 190, column 1, paragraph 3 -column 2, paragraph 2 page 191, column 2, paragraph 1	1-3,6-9, 13-16
Y	SIM B K L ET AL: "Receptor and ligand domains for invasion of erythrocytes by Plasmodium falciparum." SCIENCE (WASHINGTON D C), vol. 264, no. 5167, 1994, pages 1941-1944, XP000910268 ISSN: 0036-8075 abstract page 1942; table 1 page 1942, column 2, paragraph 1	5
Y	ADAMS J H ET AL: "A FAMILY OF ERYTHROCYTE BINDING PROTEINS OF MALARIA PARASITES" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 89, no. 15, 1992, pages 7085-7089, XP000910266 1992 ISSN: 0027-8424 abstract page 7087; figure 2 page 7088, column 1, paragraph 3 page 7089, column 1, paragraph 1 - paragraph 3	5

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	NARUM D L ET AL: "Inhibition of Plasmodium falciparum sialic acid-dependent (alternative pathway) invasion mediated by antibodies against EBA-175 region II." AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE, vol. 61, no. 3 SUPPL., 1999, pages 205-206, XP000916077 48th Annual Meeting of the American Society of Tropical Medicine and Hygiene;Washington, D.C., USA; November 28-December 2, 1999 ISSN: 0002-9637 abstract ---	1,2,6-9
P,X	LUU T ET AL: "Epitope mapping of monoclonal antibodies that block the binding of the Plasmodium falciparum merozoite ligand EBA-175." AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE, vol. 61, no. 3 SUPPL., 1999, pages 492-493, XP000916076 48th Annual Meeting of the American Society of Tropical Medicine and Hygiene;Washington, D.C., USA; November 28-December 2, 1999 ISSN: 0002-9637 abstract -----	1,2,6-9, 11,12

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/05820

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9640766 A	19-12-1996	US 5993827 A	30-11-1999
		AU 720355 B	01-06-2000
		AU 6160596 A	30-12-1996
		CA 2223512 A	19-12-1996
		EP 0832118 A	01-04-1998
WO 9507353 A	16-03-1995	AU 694142 B	16-07-1998
		AU 7872194 A	27-03-1995
		CA 2171193 A	16-03-1995
		CN 1134173 A	23-10-1996
		EP 0719333 A	03-07-1996
		JP 9502353 T	11-03-1997
		US 5993827 A	30-11-1999
		US 5849306 A	15-12-1998

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
05213-0463WPks219647		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/05820	03/03/2000	04/03/1999
International Patent Classification (IPC) or national classification and IPC C07K16/20		
Applicant ENTREMED, INC. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☒	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
VI	☐	Certain documents cited
VII	☒	Certain defects in the international application
VIII	☒	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
29/09/2000	08.06.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Montrone, M Telephone No. +49 89 2399 8711

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/05820

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-24 as originally filed

Claims, No.:

1-16 as originally filed

Drawings, sheets:

1/3-3/3 as originally filed

Sequence listing part of the description, pages:

1,2, filed with the letter of 03.03.2000

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

☐ the description, pages:

☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

iii. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 8(part),10, 13 to 16.

because:

☒ the said international application, or the said claims Nos. 8(part),10, 13 to 16 with respect to IA relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	5
	No:	Claims	1-4,6-16
Inventive step (IS)	Yes:	Claims	5
	No:	Claims	1-4,6-16
Industrial applicability (IA)	Yes:	Claims	1-7,9,11,12
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Reference is made to the following documents:

D1: WO-A-9640766
D2: WO-A-9507353
D3: Molecular and Biochemical Parasitology, vol. 95, 1998, p.: 183-192
D4: Science, vol. 264, 1994, p.: 1941-1944

Item III:

Claims 8 (partially), 10 and 13 to 16 (completely) relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(i) PCT).

Item V:

1. Claim 1 refers to an isolated antibody which binds to a 5' cysteine rich region of an EBA-175 protein from Plasmodium species.

   D1 discloses polypeptides of the Duffy Antigen Binding Protein (DABP) and the Sialic Acid Binding Protein (SABP) and their respective 5' cysteine rich regions which are identical to the EBA-175 protein of present claim 1 (see page 1, lines 11 to 31; page 2, lines 2 to 9 and line 32 to page 3, line 3; Fig. 1; page 6, lines 20 to 36). In addition, the SABP is considered to be identical to the EBA-175 protein of the present application. Conserved polypeptides of the region II/F2 of the EBA-175/SABP protein are disclosed (see page 5, lines 1 to 5; page 7, lines 17 to 19 and fig. 1). A conserved sequence of 15 amino acids in the region II/F2 of the EBA-175 protein family is disclosed which is considered to fall under the scope of protection of present claim 4 due to the broad and unclear wording of "approximately 10 amino acids" (see fig. 1, lines 1 to 10). Moreover, monoclonal and polyclonal or recombinant parts of antibodies directed against said polypeptides, hybridomas and methods for inhibiting binding and therefore invasion of merozoites into erythrocytes are taught (see page

7, line 20 to page 8, line 5; page 9, lines 10 to 19 and page 15, lines 31 to 35). Furthermore, methods for treating patients already suffering from a malaria infection with said antibodies are disclosed (see page 19, lines 21 to 31). Thus, D1 is considered to be detrimental to the novelty of the subject-matter of claims 1 to 4 and 6 to 16.

D2 discloses the same subject-matter like D1 (see page 5, lines 12 to 22; fig. 1; page 6, line 22 to page 7, line 17; page 9, line 25; page 11, lines 7 to 37; page 12, line 10 to page 13, line 12; page 25, lines 4 to 12 and page 27, line 30 to page 28, line 10). Therefore, D2 is considered to be detrimental to the novelty of the subject-matter of claims 1 to 4 and 6 to 16.

D3 discloses the delineation of functional regions on the EBA-175 protein and antibodies directed against said regions. Region II/F2 is mentioned (see abstract, table 1; fig. 1; page 188, left col., item 3.1; page 190, left col., third para. to right col., second para.). The antisera produced against said epitope are used in a merozoite invasion inhibition assay (see page 189, left col., item 3.5). Consequently, D3 is considered to be detrimental to the novelty of the subject-matter of claims 1 to 3, 6 to 9 and 13 to 16.

Thus, the subject-matter of claims 1 to 4 and 6 to 16 is not considered to be novel and does not comply with the provisions of Article 33(2) PCT.

2. The sequence of claim 5 is not explicitly disclosed in the above mentioned prior art. Thus, it is considered to be novel and complies with the provisions of Article 33(2) PCT.

3. Moreover, the subject-matter of claim 5 appears to be inventive for the following reasons:

D1 is considered to be the closest prior art. Said document discloses antibodies which bind to the region II/F2 of the EBA-175 protein family. In addition, D1 discloses six conserved amino acid sequence parts in the region II/F2 of the EBA-175 protein which consists of over 300 amino acids. The first conserved sequence block consists of 15 amino acids which include the 10 amino acid sequence of SEQ ID NO: 1 (see

fig. 1 of D1). The subject-matter of claim 5 is distinguished therefrom by selecting the 10 amino acid sequence of SEQ ID NO: 1 from the 300 amino acid region II/F2 of the EBA-175 protein for producing an antibody which inhibits the merozoite invasion into erythrocytes.

The objective problem to be solved by the present application was therefore to further characterise an epitope which elicits antibodies for specifically inhibiting the merozoite invasion into red blood cells (RBC).

It was already known that the 300 amino acid II/F2 region of the EBA-175 protein was responsible for binding to the glycophorin A receptor on RBCs and therefore a target for a receptor blocking approach (see D4, abstract; table 1; page 1942, middle col., first para.;). However, it was nowhere taught in the prior art that antibodies raised against the particular sequence of SEQ ID NO: 1 would result in a specific inhibition of merozoite invasion into RBCs. Thus, the selection of said 10 amino acids for raising antibodies against it resulted in an unexpected technical effect. Consequently, the subject-matter of claim 5 appears to be inventive and does comply with the provisions of Article 33(3) PCT.

3. For the assessment of the present claims 8 (partially), 10 and 13 to 16 (completely) on the question whether they are industrially applicable, no unified criteria exist in the PCT. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Item VI:

The documents US-A-5993827 filed at the 07.06.1995, published at 30.11.1999 and claiming the priority of 07.09.1994, American Journal of Tropical Medicine and Hygiene, Sept. 1999, vol. 61, p.: 492-493 and American Journal of Tropical Medicine and Hygiene, Sept. 1999, vol. 61, p.: 205-206 could be relevant to the subject-matter of the present application if the priority of the claims is not valid.

Item VII:

1. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1 to D4 is not mentioned in the description, nor are these documents identified therein.

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C07K 16/20, C12N 5/20, A61K 39/395, A61P 33/06

A2

(11) International Publication Number: WO 00/52056

(43) International Publication Date: 8 September 2000 (08.09.00)

(21) International Application Number: PCT/US00/05820

(22) International Filing Date: 3 March 2000 (03.03.00)

(30) Priority Data:

60/122,842	4 March 1999 (04.03.99)	US
60/153,575	13 September 1999 (13.09.99)	US

(71) Applicant *(for all designated States except US)*: ENTREMED, INC. [US/US]; 9640 Medical Center Drive, Rockville, MD 20850 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: NARUM, David, L. [US/US]; 8533 Fountain Valley Drive, Gaithersburg, MD 20879 (US). SIM, Kim, Lee [US/US]; 308 Argosy Drive, Gaithersburg, MD 20878 (US).

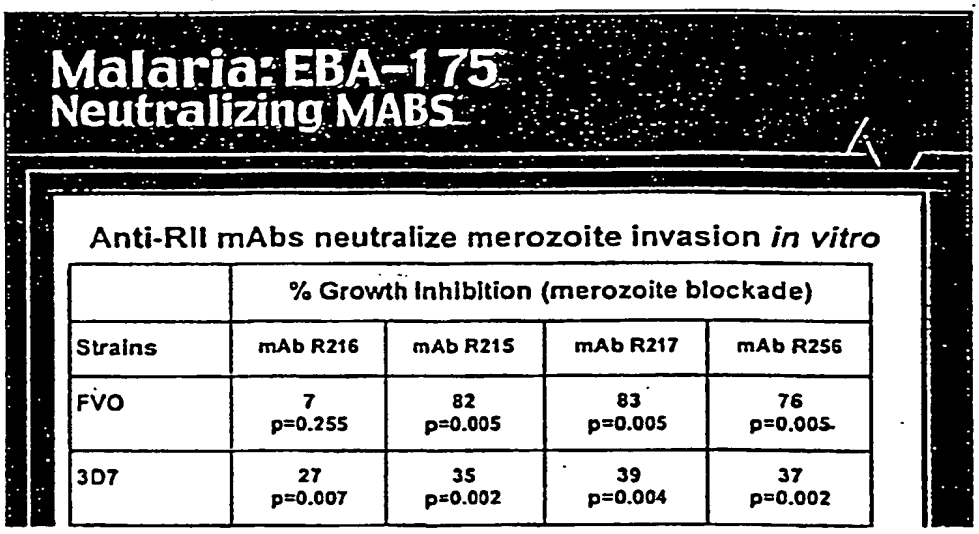
(74) Agents: ELSEVIER, Lisa, C. et al.; Jones & Askew, LLP, 2400 Monarch Tower, 3424 Peachtree Road, N.E., Atlanta, GA 30326 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

Without international search report and to be republished upon receipt of that report.

(54) Title: ANTI-PLASMODIUM COMPOSITIONS AND METHODS OF USE



(57) Abstract

Compositions that inhibit the binding of *Plasmodium falciparum* to erythrocytes are provided. More particularly, antibodies specific for *Plasmodium falciparum* binding proteins and blocking peptides that prevent the binding of *Plasmodium falciparum* are included in the present invention. The methods provided utilize the antibody and peptide compositions provided herein and include methods for the diagnosis, prevention, and treatment of *Plasmodium falciparum* diseases such as malaria as well as methods for the detection of *Plasmodium falciparum* in biological samples and culture media.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 September 2000 (08.09.2000)

PCT

(10) International Publication Number
WO 00/52056 A3

(51) International Patent Classification[7]: C07K 16/20, C12N 5/20, A61K 39/395, A61P 33/06

(21) International Application Number: PCT/US00/05820

(22) International Filing Date: 3 March 2000 (03.03.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/122,842 4 March 1999 (04.03.1999) US
60/153,575 13 September 1999 (13.09.1999) US

(71) Applicant *(for all designated States except US)*: ENTREMED, INC. [US/US]; 9640 Medical Center Drive, Rockville, MD 20850 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: NARUM, David, L. [US/US]; 8533 Fountain Valley Drive, Gaithersburg, MD 20879 (US). SIM, Kim, Lee [US/US]; 308 Argosy Drive, Gaithersburg, MD 20878 (US).

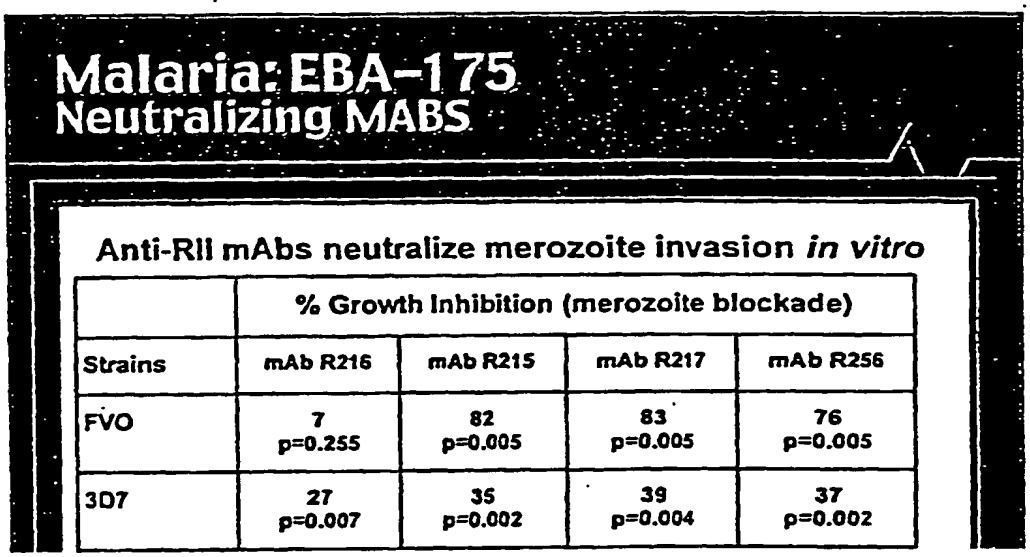
(74) Agents: ELSEVIER, Lisa, C. et al.; Jones & Askew, LLP, 2400 Monarch Tower, 3424 Peachtree Road, N.E., Atlanta, GA 30326 (US).

(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: ANTI-PLASMODIUM COMPOSITIONS AND METHODS OF USE



(57) Abstract: Compositions that inhibit the binding of *Plasmodium falciparum* to erythrocytes are provided. More particularly, antibodies specific for *Plasmodium falciparum* binding proteins and blocking peptides that prevent the binding f *Plasmodium falciparum* are included in the present invention. The methods provided utilize the antibody and peptide compositions provided herein and include methods for the diagnosis, prevention, and treatment of *Plasmodium falciparum* diseases such as malaria as well as methods for the detection of *Plasmodium falciparum* in biological samples and culture media.

WO 00/52056 A3

Published:
— *With international search report.*

(88) Date f publication f the international search report:
21 December 2000

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/05820

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K16/20 C12N5/20 A61K39/395 A61P33/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, EPO-Internal, WPI Data, PAJ, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 40766 A (US HEALTH) 19 December 1996 (1996-12-19) abstract page 1, line 11-31 page 2, line 2 page 2, line 32 -page 3, line 3 page 6, line 20 - line 36 page 5, line 1 - line 5 page 7, line 17 - line 19 page 7, line 20 -page 8, line 5 page 9, line 10 - line 19 page 15, line 31 - line 35 figure 1 --- -/--	1-4,6-16

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 August 2000	24.08.00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Montrone, M

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED T BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 07353 A (US HEALTH) 16 March 1995 (1995-03-16) abstract page 5, line 12 - line 22 page 6, line 22 -page 7, line 17 page 9, line 25 page 11, line 7 - line 37 page 12, line 10 -page 13, line 12 page 25, line 4 - line 12 page 27, line 30 -page 28, line 10	1-4,6-16
X	B. KIM LEE SIM: "Delineation of functional regions on Plasmodium falciparum EBA-175 by antibodies eluded from immune complexes" MOL. BIOCHEM. PARASITOLOGY, vol. 95, 1998, pages 183-192, XP000915150 abstract page 184; table 1 page 186, column 2, paragraph 5 page 187; figure 1 page 188, column 1, paragraph 4 page 189, column 1, paragraph 3 -column 2, paragraph 1 page 190, column 1, paragraph 3 -column 2, paragraph 2 page 191, column 2, paragraph 1	1-3,6-9, 13-16
Y	SIM B K L ET AL: "Receptor and ligand domains for invasion of erythrocytes by Plasmodium falciparum." SCIENCE (WASHINGTON D C), vol. 264, no. 5167, 1994, pages 1941-1944, XP000910268 ISSN: 0036-8075 abstract page 1942; table 1 page 1942, column 2, paragraph 1	5
Y	ADAMS J H ET AL: "A FAMILY OF ERYTHROCYTE BINDING PROTEINS OF MALARIA PARASITES" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 89, no. 15, 1992, pages 7085-7089, XP000910266 1992 ISSN: 0027-8424 abstract page 7087; figure 2 page 7088, column 1, paragraph 3 page 7089, column 1, paragraph 1 - paragraph 3	5

-/—

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	NARUM D L ET AL: "Inhibition of Plasmodium falciparum sialic acid-dependent (alternative pathway) invasion mediated by antibodies against EBA-175 region II." AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE, vol. 61, no. 3 SUPPL., 1999, pages 205-206, XP000916077 48th Annual Meeting of the American Society of Tropical Medicine and Hygiene;Washington, D.C., USA; November 28-December 2, 1999 ISSN: 0002-9637 abstract	1,2,6-9
P,X	LUU T ET AL: "Epitope mapping of monoclonal antibodies that block the binding of the Plasmodium falciparum merozoite ligand EBA-175." AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE, vol. 61, no. 3 SUPPL., 1999, pages 492-493, XP000916076 48th Annual Meeting of the American Society of Tropical Medicine and Hygiene;Washington, D.C., USA; November 28-December 2, 1999 ISSN: 0002-9637 abstract	1,2,6-9, 11,12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/05820

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 8 (partially), 10 and 13 to 16 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intl. Application No
PCT/US 00/05820

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9640766 A	19-12-1996	US 5993827 A	30-11-1999
		AU 720355 B	01-06-2000
		AU 6160596 A	30-12-1996
		CA 2223512 A	19-12-1996
		EP 0832118 A	01-04-1998
WO 9507353 A	16-03-1995	AU 694142 B	16-07-1998
		AU 7872194 A	27-03-1995
		CA 2171193 A	16-03-1995
		CN 1134173 A	23-10-1996
		EP 0719333 A	03-07-1996
		JP 9502353 T	11-03-1997
		US 5993827 A	30-11-1999
		US 5849306 A	15-12-1998

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

ANTI-PLASMODIUM COMPOSITIONS AND METHODS OF USE

Cross Reference to Related Applications

This application claims priority to U.S. Provisional Patent Application Serial No. 60/122,842, filed March 4, 1999 and U.S. Provisional Patent Application Serial No. 60/153,575, filed September 13, 1999.

Field of the Invention

The present invention relates to the fields of microbiology and immunology and more specifically relates to compositions and methods for the detection, diagnosis, and treatment of malaria. In particular, the invention pertains to peptides and antibodies that inhibit the binding of *Plasmodium falciparum* erythrocyte binding protein antigens to erythrocytes.

Background of the Invention

Although endemic malaria has disappeared from the United States, malaria continues to be one of the most important infectious diseases in the world as it kills millions of people each year in countries throughout Africa, Asia and Latin America. The characteristic presentation of malaria is chills followed by a fever ranging from 104-107° F, followed by profuse sweating. Other manifestations of malaria include anemia, decreased blood flow to vital organs, thrombocytopenia, and glomerulonephritis. Additionally, when the

central nervous system is involved, symptoms include delirium, convulsions, paralysis, coma, and even rapid death.

Malarial diseases in humans are caused by four species of the *Plasmodium* parasite: *P. falciparum*, *P. vivax*, *P. ovali*, and *P. malariae*. Each of these species is transmitted to the human via a female *Anopheles* mosquito that transmits *Plasmodium* parasites, or sporozoites. Once the sporozoites enter the bloodstream of the human, they localize in liver cells, or hepatocytes. One to two weeks later, the infected hepatocytes rupture and release mature parasites, or merozoites. The merozoites then begin the erythrocytic phase of malaria by attaching to and invading red blood cells, or erythrocytes.

The invasion of the erythrocytes by the malarial parasites is the direct cause of malarial pathogenesis and pathology. The fever, anemia, circulatory changes, and immunopathologic phenomena characteristic of malaria are largely the result of red cell rupture and the host's immune response to parasitized erythrocytes. For these reasons, the erythrocytic stage of the *Plasmodium* life cycle is of vital importance to vaccine development and treatment of malaria.

There are a number of strategies for developing new or novel therapeutics for the erythrocytic stage of malaria. One strategy is to identify parasitic molecules that are critical to the survival of the parasite. Extracellular merozoites released from infected hepatocytes or from infected erythrocytes must invade other erythrocytes within minutes if they are to survive. Invasion by the malaria parasite is dependent upon the binding of parasite proteins to receptors on the erythrocyte surface (Hadley et al., 1986).

Interestingly, different parasite species use different erythrocytic receptors for invasion of erythrocytes. *P. falciparum* invades erythrocytes through a 175 kDa erythrocyte binding protein called EBA-175. EBA-175 functions as an erythrocyte invasion ligand that binds to its receptor, glycophorin A, on erythrocytes during invasion (Camus and Hadley, 1985; Sim *et al.*, 1990; Orlandi *et al.*, 1992; Sim *et al.*, 1994b). In contrast, the human *P. vivax* and the simian *P. knowlesi* invade erythrocytes by binding Duffy blood group antigens present on some erythrocytes (Miller *et al.*, 1975). The genes encoding the Duffy antigen binding proteins of *P. vivax* and *P. knowlesi* have been cloned and sequenced (Fang *et al.*, 1991 and Adams *et al.*, 1990, respectively).

Sequencing of the genes encoding the proteins used by *P. vivax* and *P. knowlesi* for erythrocyte invasion demonstrated that these proteins are members of the same gene family as the genes that encode the EBA-175, the protein used by *P. falciparum* for erythrocyte invasion (Adams *et al.*, 1992). Homology between the Duffy binding proteins and EBA-175 is restricted to 5' and 3' cysteine rich domains. Within these cysteine rich domains, the cysteines and some aromatic residues are conserved, but the intervening amino acid sequences differ. Sim *et al.* (1994b) demonstrated that the 5' cysteine rich domain of EBA-175 of *P. falciparum* contains the receptor binding domain, while Chitnis and Miller (1994) demonstrated that the 5' cysteine rich region of *P. vivax* and *P. knowlesi* contain the Duffy binding domain. See Figure 1.

Summary of the Invention

The present invention provides compositions and methods for detecting, diagnosing, and treating *Plasmodium* and *Plasmodium* related infections. In particular, the compositions include EBA-175 antibodies and peptides that specifically inhibit binding of *Plasmodium* erythrocyte binding proteins to erythrocytes. Preferably, the antibodies specifically bind to the EBA-175 erythrocyte binding protein of *P. falciparum*. In a further preferred embodiment, the antibodies are designated R215, R215, R217 and R256 and have the properties as described herein. Additionally, in another preferred embodiment, EBA-175 peptides have the amino acid sequences of SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:3.

The EBA-175 antibodies of the present invention can be polyclonal antibodies or monoclonal antibodies. Antibodies specific for EBA-175 may be administered to a human or animal to passively immunize the human or animal against *P. falciparum* infection, thereby reducing *P. falciparum* related diseases such as malaria. EBA-175 peptides specific for *P. falciparum* erythrocyte binding proteins may be administered to a human or animal to immunize the human or animal against *P. falciparum* infection, thereby reducing *P. falciparum* related diseases such as malaria. The antibodies are also useful as *in vitro* research tools for studying malaria and for isolating large quantities of EBA-175 proteins. The EBA-175 antibodies can be used in diagnostic kits to detect the presence and quantity of *P. falciparum*, which is diagnostic or prognostic for the occurrence or recurrence of diseases such as malaria. Additionally, the EBA-175 peptides that inhibit binding of *P.*

falciparum binding to erythrocytes can be used in diagnostic kits to detect the presence and quantity of *P. falciparum* antibodies, which is diagnostic or prognostic for the occurrence of diseases such as malaria.

The compositions also include *P. falciparum* antibodies and antibody fragments, *P. falciparum* blocking peptides, *P. falciparum* antisera, *P. falciparum* receptor agonists and *P. falciparum* receptor antagonists linked to cytotoxic agents. Such compositions are useful for research applications. The compositions, when combined with pharmaceutically acceptable excipients, or sustained-release compounds or compositions, such as biodegradable polymers, are useful as therapeutic agents such as vaccine or treatment compositions.

Diagnostic and analytical methods and kits may be developed for detection and measurement of *P. falciparum* in a variety of biological samples including biological fluids and biological tissues, and for localization of *P. falciparum* in tissues and cells. The method and kit can be in any configuration well known to those of ordinary skill in the art.

The methods of the present invention include methods of treating, diagnosing and preventing *P. falciparum* diseases such as malaria. Also provided are methods of detecting EBA-175 protein, *P. falciparum* and *P. falciparum* erythrocyte binding proteins. These methods employ the EBA-175 antibodies and peptides described herein. Methods of prevention may include passive immunization prior to infection by *P. falciparum* parasites to inhibit parasitic infection of erythrocytes. Methods of treatment may also include administration after infection to inhibit the spread of the parasite and ameliorate the symptoms of *P. falciparum* infection. Methods of diagnosis of *P. falciparum* infection include methods directed toward combining a biological sample with the antibodies described herein, wherein the binding of the antibodies indicates malaria. Methods of detection of *P. falciparum* and *P. falciparum* erythrocyte binding proteins include methods directed toward the detection of EBA-175 protein, *P. falciparum* and *P. falciparum* erythrocyte binding proteins in biological samples such as biological fluids and tissues and in culture media.

Accordingly, it is an object of the present invention to provide compositions comprising one or more EBA-175 antibodies or blocking peptides.

It is another object of the present invention to provide a method for the treatment of *P. falciparum* related diseases such as malaria.

It is a further object of the present invention to provide a method for the treatment of malaria, wherein compositions comprising one or more EBA-175 antibodies and/or blocking peptides are administered to an individual in need of such treatment.

It is another object of the present invention to provide a method for the diagnosis of *P. falciparum* related diseases such as malaria.

It is yet another object of the present invention to provide a method for the diagnosis of malaria, wherein compositions comprising one or more EBA-175 antibodies are used.

A further object of the present invention is to provide a method for the prevention of *P. falciparum* related diseases such as malaria.

It is another object of the present invention to provide a method for the prevention of malaria, wherein compositions comprising one or more EBA-175 antibodies or peptides are administered to an individual in need of such prevention.

Another object of the present invention to provide a method of detection of *P. falciparum* in culture media and in biological samples such as biological tissues and fluids.

It is a further object of the present invention to provide a method of detection of *P. falciparum*, wherein compositions comprising one or more EBA-175 antibodies are used.

These and other objects, features and advantages of the present invention will become apparent after a review of the following detailed description of the disclosed embodiments and the appended claims.

Brief Description of the Figures

Figure 1: *Gene Structure of P. Erythrocyte Binding Proteins*

Region I of EBA-175 encompasses amino acid residues 20-157, region II amino acids 145-760, region III-V amino acids 743-1322 and region VI amino acids 1304-1394. Region II is further subdivided into regions F1 and F2.

Figure 2: *Phage Sequence of two EBA-175 Blocking Peptides*

Following panning against a constrained heptapeptide M13 phage display library using EBA-175 antibody R256, the amino acid sequence

of several peptides was determined. Six of eleven peptides contained the sequence of PEP R256-2, while one of the peptides contained the sequence of PEP R256-1.

Figure 3: *Percentage of P. falciparum growth inhibition by EBA-175 Monoclonal Antibodies*

Monoclonal antibodies R215, R216, R217 and R256 were tested for their ability to neutralize merozoite invasion *in vitro*. As compared to control monoclonal antibodies, R215, R217 and R256 blocked 82%, 83% and 76%, respectively, of merozoite invasion in the homologous FVO strain. Additionally, R215, R217 and R256, blocked 35%, 39% and 37%, respectively, of merozoite invasion in the heterologous 3D7 strain.

Detailed Description

Compositions and methods for treating *P. falciparum* infection, diagnosing diseases related to *P. falciparum* infection, and preventing diseases related to *P. falciparum* infection are provided. The compositions are isolated antibodies and peptides that inhibit binding of *P. falciparum* erythrocyte binding proteins and fragments thereof. The antibodies and peptides of the present invention bind to an EBA-175 erythrocyte binding protein of a *Plasmodium* species. In one embodiment of the present invention, the *Plasmodium* species is *P. falciparum* and the EBA-175 protein is that as described in Camus and Hadley (1985), Sim *et al.* (1990), and Orlandi *et al.* (1992).

As used herein, the terms "antibody" and "antibodies" include monoclonal antibodies, polyclonal, chimeric, single chain, bispecific, simianized, and humanized antibodies, Fab fragments, including the products of an Fab immunoglobulin expression library, and peptide antibody fragments. Also as used herein, the terms "EBA-175 antibody" or "EBA-175 antibodies" refer to antibodies that bind to an EBA-175 erythrocyte binding protein of a *Plasmodium* species. It is also to be understood that as used herein, the term "isolated" refers to a composition which is substantially or essentially free from at least some of the components that normally accompany it in its native state. Thus, the isolated EBA-175 antibodies and proteins of this invention do not contain some of the materials normally associated with their *in situ* environment. Typically, the isolated EBA-175 antibodies and proteins of the invention are at

least about 80% pure, usually at least about 90% pure, and preferably at least about 95% pure as measured by band intensity on a silver stained gel.

It is to be understood that the term "isolated" does not exclude fusion proteins comprising the EBA-175 antibodies and proteins from the present invention. The present invention contemplates fusion proteins, or chimeric proteins, comprising the EBA-175 antibodies and proteins described herein and other proteins. The present invention also includes humanized EBA-175 antibodies. For example, EBA-175 antibodies described herein that are of murine origin may be humanized by methods known to those of skill in the art. Examples of methods used to humanize antibodies of non-human origin are the use of transgenic mice having genes encoding human immunoglobulins and the use of recombinant technology to create a fusion protein comprising the variable region domains of the non-human antibodies and human immunoglobulin genes. Therefore, the present invention contemplates humanized EBA-175 antibodies comprising the variable regions of the antibodies described herein and the non-variable regions of human immunoglobulin, and in particular, human IgG.

Additionally, the terms "a", "an" and "the" as used herein are defined to mean "one or more" and include the plural unless the context is inappropriate. The terms "polypeptide", "peptide", and "protein", as used herein, are interchangeable and are defined to mean a biomolecule composed of two or more amino acids linked by a peptide bond.

The present invention includes antibodies and peptides that specifically bind to an EBA-175 erythrocyte binding protein of a *Plasmodium* species. As used herein, the terms "specifically bind" and "selectively bind" are synonymous and refer to a binding reaction in which, under designated immunoassay conditions, the specified antibodies and peptides bind preferentially to a particular peptide/protein or antibody, respectively, and do not bind in a significant amount to other peptides/proteins or antibodies present in the sample. The specific binding is determinative of the presence of a peptide or antibody in the presence of a heterogeneous population of proteins and other biologics. Specific binding to a peptide or antibody under such conditions requires an antibody or blocking peptide that is selected for its specificity for a particular protein. A variety of immunoassay formats may be used to select antibodies and peptides specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select

monoclonal antibodies specifically immunoreactive with a protein. *See*, Harlow and Lane (1988) *Antibodies, A Laboratory Manual*, Cold Spring Harbor Publications, New York, for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity.

In one embodiment of the present invention, an antibody binds to a 5' cysteine rich region of an EBA-175 erythrocyte binding protein of a *Plasmodium* species. In a preferred embodiment, the *Plasmodium* species is *P. falciparum*. The 5' cysteine rich region of *P. falciparum* is shown in Figure 1 as Region II. Region II encompasses approximately amino acids 145-760 of the EBA-175 protein. As shown in Figure 1, the 5' cysteine rich region is further divided into two regions F1 and F2 that are designated herein as RII/F1 and RII/F2, respectively. Included in the present invention are antibodies that bind to Region II of an EBA-175 protein of *P. falciparum*, and more preferably bind to the RII/F2 region of an EBA-175 protein of *P. falciparum*.

In one embodiment of the present invention, an antibody is provided that binds to a region of an EBA-175 protein, wherein the region consists of an approximately 10 amino acid sequence that is a fragment of an RII/FII region of an EBA-175 protein. In a further embodiment, the 10 amino acid sequence corresponds to approximately amino acids 488-497 of an EBA-175 protein, and more preferably has the amino acid sequence of CVPPRRQELC (SEQ ID NO:1).

The compositions of the present invention also include peptides that inhibit binding of a *Plasmodium* species and/or EBA-175 proteins or peptides to erythrocytes. As used herein, the term "EBA-175 peptide" refers to a 5-100 amino acid sequence of an EBA-175 protein of a *Plasmodium* species, more preferably a 5-25 amino acid sequence of an EBA-175 protein of a *Plasmodium* species, and most preferably a 5-15 amino acid sequence of an EBA-175 protein of a *Plasmodium* species. In one embodiment, the peptides inhibit binding of the erythrocyte binding protein EBA-175 as defined by Camus and Hadley (1985), Sim *et al.* (1990), and Orlandi *et al.* (1992). In another embodiment, the present invention provides an EBA-175 peptide that corresponds to approximately amino acids 488-497 of an EBA-175 protein. In a preferred embodiment, the peptides have the amino acid sequences of CVPPRRQELC (SEQ ID NO:1), CNMVPMSRC (SEQ ID NO:2) or CWSINPRWC (SEQ ID NO:3). When creating the peptides of the present invention, it is to be noted that the peptides may optionally comprise a carboxy-

terminal amino acid sequence of GGGS (SEQ ID NO:4) as is well known in phage display techniques.

The terms "corresponding to" and "corresponds to", when referring to amino acids, indicates the comparison of two amino acids in the same region of different proteins, or fragments thereof, wherein the proteins are homologs, orthologs or paralogs. Homologs are defined as proteins with substantial homology, wherein "substantial homology" is defined below. Orthologs are defined as proteins having non-identical amino acid sequences and similar functional characteristics, wherein the proteins are from different species, but wherein the species have a common ancestral origin. Paralogs are defined as proteins having non-identical amino acid sequences and similar functional characteristics, wherein the proteins are from the same species.

It is also to be understood that the present invention is contemplated to include any EBA-175 peptide derivative. An EBA-175 peptide derivative includes a protein having an amino acid sequence of an EBA-175 peptide. An EBA-175 peptide derivative also includes a peptide having a sequence corresponding to an EBA-175 peptide subsequence. A "subsequence" is a sequence of contiguous amino acids found within a larger sequence. As defined herein, a larger sequence is an EBA-175 peptide of approximately 10 amino acids. A subsequence is generally composed of approximately at least 70%, more preferably 80%, and most preferably 90% of the larger sequence.

An EBA-175 peptide derivative also includes a peptide having a modified sequence in which one or more amino acids in the original sequence or subsequence have been substituted with a naturally occurring amino acid residue or amino acid residue analog (also referred to as modified amino acid). Suitable EBA-175 peptide derivatives have modified sequences which are substantially homologous to the amino acid sequence of an EBA-175 peptide or to an antiangiogenic subsequence of a EBA-175 peptide.

An "amino acid residue" is a moiety found within a protein or peptide and is represented by -NH-CHR-CO-, wherein R is the side chain of a naturally occurring amino acid. When referring to a moiety found within a peptide, the terms "amino acid residue" and "amino acid" are used interchangeably. An "amino acid residue analog" includes D or L configurations having the following formula: -NH-CHR-CO-, wherein R is an aliphatic group, a substituted aliphatic aromatic group, a benzyl group, a

substituted benzyl group, an aromatic group or a substituted aromatic group and wherein R does not correspond to the side chain of a naturally occurring amino acid.

Suitable substitutions for amino acid residues in the sequence of the EBA-175 peptides described herein include conservative substitutions that result in EBA-175 peptides that block *P. falciparum* or EBA-175 protein binding to erythrocytes. A conservative substitution is a substitution in which the substituting amino acid (naturally occurring or modified) is structurally related to the amino acid being substituted. "Structurally related" amino acids are approximately the same size and have the same or similar functional groups in the side chains.

Provided below are groups of naturally occurring and modified amino acids in which each amino acid in a group has similar electronic and steric properties. Thus, a conservative substitution can be made by substituting an amino acid with another amino acid from the same group. It is to be understood that these groups are non-limiting and that additional modified amino acids could be included in each group.

Group I includes leucine, isoleucine, valine, methionine and modified amino acids having the following side chains: ethyl, *n*-propyl *n*-butyl. Preferably, Group I includes leucine, isoleucine, valine and methionine.

Group II includes glycine, alanine, valine and a modified amino acid having an ethyl side chain. Preferably, Group II includes glycine and alanine.

Group III includes phenylalanine, phenylglycine, tyrosine, tryptophan, cyclohexylmethyl, and modified amino residues having substituted benzyl or phenyl side chains. Preferred substituents include one or more of the following: halogen, methyl, ethyl, nitro, $-NH_2$, methoxy, ethoxy and –CN. Preferably, Group III includes phenylalanine, tyrosine and tryptophan.

Group IV includes glutamic acid, aspartic acid, a substituted or unsubstituted aliphatic, aromatic or benzylic ester of glutamic or aspartic acid (e.g., methyl, ethyl, *n*-propyl *iso*-propyl, cyclohexyl, benzyl or substituted benzyl), glutamine, asparagine, -CO-NH-alkylated glutamine or asparagine (e.g., methyl, ethyl, *n*-propyl and *iso*-propyl) and modified amino acids having the side chain $-(CH_2)_3$-COOH, an ester thereof (substituted or unsubstituted aliphatic, aromatic or benzylic ester), an amide thereof and a substituted or unsubstituted N-alkylated amide thereof. Preferably, Group IV includes

glutamic acid, aspartic acid, methyl aspartate, ethyl aspartate, benzyl aspartate and methyl glutamate, ethyl glutamate and benzyl glutamate, glutamine and asparagine.

Group V includes histidine, lysine, ornithine, arginine, N-nitroarginine, β-cycloarginine, γ-hydroxyarginine, N-amidinocitruline and 2-amino-4-guanidinobutanoic acid, homologs of lysine, homologs of arginine and homologs of ornithine. Preferably, Group V includes histidine, lysine, arginine and ornithine. A homolog of an amino acid includes from 1 to about 3 additional or subtracted methylene units in the side chain.

Group VI includes serine, threonine, cysteine and modified amino acids having C1-C5 straight or branched alkyl side chains substituted with –OH or –SH, for example, $-CH_2CH_2OH$, $-CH_2CH_2CH_2OH$ or $-CH_2CH_2OHCH_3$. Preferably, Group VI includes serine, cysteine or threonine.

In another aspect of the present invention, suitable substitutions for amino acid residues in the amino acid sequences described herein include "severe substitutions" that result in EBA-175 derivatives that block *P. falciparum* or EBA-175 protein binding to erythrocytes. Severe substitutions that result in EBA-175 derivatives that block *P. falciparum* or EBA-175 protein binding to erythrocytes are much more likely to be possible in positions that are not highly conserved than at positions that are highly conserved. In the present invention, severe substitutions are much less likely to be possible in the conserved cysteine amino acid residues. A "severe substitution" is a substitution in which the substituting amino acid (naturally occurring or modified) has significantly different size and/or electronic properties compared with the amino acid being substituted. For example, the side chain of the substituting amino acid can be significantly larger (or smaller) than the side chain of the amino acid being substituted and/or can have functional groups with significantly different electronic properties than the amino acid being substituted.

Examples of severe substitutions of this type include the substitution of phenylalanine or cyclohexylmethyl glycine for alanine, isoleucine for glycine, a D amino acid for the corresponding L amino acid or –NH-CH[$(-CH_2)_5$-COOH]-CO- for aspartic acid. Alternatively, a functional group may be added to the side chain, deleted from the side chain or exchanged with another functional group. Examples of severe substitutions of this type include adding an amine or hydroxyl, carboxylic acid to the aliphatic side chain of valine,

leucine or isoleucine, exchanging the carboxylic acid in the side chain of aspartic acid or glutamic acid with an amine or deleting the amine group in the side chain of lysine or ornithine. In yet another alternative, the side chain of the substituting amino acid can have significantly different steric and electronic properties that the functional group of the amino acid being substituted. Examples of such modifications include tryptophan for glycine, lysine for aspartic acid and $-(CH_2)_4COOH$ for the side chain of serine. These examples are not meant to be limiting.

"Substantial homology" exists between two amino acid sequences when a sufficient number of amino acid residues at corresponding positions of each amino acid sequence are either identical or structurally related such that a protein or peptide having the first amino acid sequence and a protein or peptide having the second amino acid sequence exhibit similar biological activities. Generally, there is substantial sequence homology among the amino acid sequences when at least 30%, more preferably at least 40%, and most preferably at least 50%, of the amino acids in the first amino acid sequence are identical to or structurally related to the second amino acid sequence. Homology is often measured using sequence analysis software, e.g., BLASTIN or BLASTP. The default parameters for comparing the two sequences (e.g., "Blast"-ing two sequences against each other) by BLASTIN (for nucleotide sequences) are reward for match =1, penalty for mismatch = -2, open gap = 5, and extension gap = 2. When using BLASTP for protein sequences, the default parameters are reward for match = 0, penalty for mismatch = 0, open gap = 11, and extension gap = 1.

The EBA-175 antibodies and peptides described herein are useful for the production of vaccines and therapeutic compositions. As used herein, the term "vaccine" includes compositions comprising the EBA-175 antibodies, or fragments thereof, as described herein, used for passive immunization of individuals prior to or following infection by *P. falciparum*. The term "vaccine" also includes compositions comprising the EBA-175 peptides described herein, used for active immunization of individuals prior to infection by *P. falciparum*. Accordingly, the present invention includes antibodies that inhibit binding of an EBA-175 protein to a red blood cell or erythrocyte. Additionally included in the present invention are antibodies that inhibit *P. falciparum* invasion into a red blood cell. The antibodies inhibit *P. falciparum* invasion of a red blood cell *in vitro* and/or *in vivo*.

The EBA-175 antibodies and peptides of the present invention can be isolated from serum or synthesized by chemical or biological methods. For example, the EBA-175 antibodies and peptides can be isolated from cell culture, produced by recombinant gene expression or polypeptide synthesis, or derived by *in vitro* enzymatic catalysis of larger, encompassing polypeptides to yield EBA-175 antibodies or peptides. Recombinant techniques include gene amplification from DNA sources using amplification techniques such as the polymerase chain reaction (PCR), and gene amplification from RNA sources using amplification techniques such as reverse transcriptase/PCR. In one embodiment, the EBA-175 peptides are produced and analyzed via phage display technology. Phage vectors that may be used in phage display technology include, but are not limited to, λ, M13, MS2, Mu, P4, λgtII, and ϕX174.

The EBA-175 antibodies and peptides of the present invention may be labeled directly with a detectable label for detection or identification of *P. falciparum* or antibody thereto. As used herein, the terms "detecting" or "detection" refer to quantitatively or quantitatively determining the presence of the biomolecule under investigation. Labels for use in immunoassays are generally known to those skilled in the art and include enzymes, radioisotopes, and fluorescent, luminescent and chromogenic substances including colored particles such as colloidal gold and latex beads. Suitable immunoassays include enzyme-linked immunosorbent assays (ELISA) and radioimmunoassays.

Alternatively, the EBA-175 antibodies and peptides of the present invention may be labeled indirectly by reaction with labeled substances that have an affinity for immunoglobulin, such as protein A or G or second antibodies. When using secondary antibodies, a suitable immunoassay is an immunoblot or Western blot. Additionally, the antibody or blocking peptide may be conjugated with a second substance and detected with a labeled third substance having an affinity for the second substance conjugated to the antibody. For example, the antibody or blocking peptide may be conjugated to biotin and the antibody-biotin conjugate detected using labeled avidin or streptavidin. Similarly, the antibody or blocking peptide may be conjugated to a hapten and the antibody-hapten conjugate detected using labeled anti-hapten antibody. These and other methods of labeling antibodies and assay conjugates are well known to those skilled in the art.

When labeled with a detectable biomolecule or chemical, the EBA-175 antibodies and peptides described above are useful for purposes such as *in vivo* and *in vitro* diagnostics and laboratory research using the methods and assays described below. Various types of labels and methods of conjugating the labels to the polypeptides and antibodies are well known to those skilled in the art. Several specific labels are set forth below.

For example, the EBA-175 antibodies and peptides are conjugated to a radiolabel such as, but not restricted to, ^{32}P, ^{3}H, ^{14}C, ^{35}S, ^{125}I, or ^{131}I. Detection of a label can be by methods such as scintillation counting, gamma ray spectrometry or autoradiography. Bioluminescent labels, such as derivatives of firefly luciferin, are also useful. The bioluminescent substance is covalently bound to the polypeptide or antibody by conventional methods, and the labeled antibody is detected when an enzyme, such as luciferase, catalyzes a reaction with ATP causing the bioluminescent molecule to emit photons of light. Fluorogens may also be used as labels. Examples of fluorogens include fluorescein and derivatives, phycoerythrin, allo-phycocyanin, phycocyanin, rhodamine, and Texas Red. The fluorogens are generally detected by a fluorescence detector.

The EBA-175 antibodies and peptides can alternatively be labeled with a chromogen to provide an enzyme or affinity label. For example, the antibody can be biotinylated so that it can be utilized in a biotin-avidin reaction, which may also be coupled to a label such as an enzyme or fluorogen. Alternatively, the antibody or peptide can be labeled with peroxidase, alkaline phosphatase or other enzymes giving a chromogenic or fluorogenic reaction upon addition of substrate. Additives such as 5-amino-2,3-dihydro-1,4-phthalazinedione (also known as Luminol™) (Sigma Chemical Company, St. Louis, MO) and rate enhancers such as p-hydroxybiphenyl (also known as p-phenylphenol) (Sigma Chemical Company, St. Louis, MO) can be used to amplify enzymes such as horseradish peroxidase through a luminescent reaction; and luminogeneic or fluorogenic dioxetane derivatives of enzyme substrates can also be used. Such labels can be detected using enzyme-linked immunoassays (ELISA) or by detecting a color change with the aid of a spectrophotometer. In addition, EBA-175 antibodies and peptides may be labeled with colloidal gold for use in immunoelectron microscopy in accordance with methods well known to those skilled in the art.

The EBA-175 antibodies and peptides described herein are particularly useful for the treatment, prevention, diagnosis and detection of *P. falciparum* infections. The EBA-175 antibodies and peptides of the present invention may be used for the treatment, prevention, diagnosis or prognosis of *P. falciparum* related diseases such as malaria. Methods of prevention include passive immunization with the antibodies of the present invention prior to infection by *P. falciparum* to inhibit parasitic infection of erythrocytes. Methods of prevention also include active immunization with the blocking peptides of the present invention prior to infection by *P. falciparum* to inhibit parasitic infection of erythrocytes. Methods of treatment include administration of the antibodies and/or blocking peptides after infection to inhibit the spread of the parasite and ameliorate the symptoms of *P. falciparum* infection.

Therefore, the present invention includes a method of treating a *Plasmodium* species related disease comprising, administering to an individual an isolated EBA-175 antibody in an amount effective to treat the *Plasmodium* species related disease. In one embodiment, the *Plasmodium* species is *P. falciparum*. In a further embodiment, the *P. falciparum* related disease is malaria. Also included in the present invention are methods of inhibiting *P. falciparum* invasion into a red blood cell of an individual comprising, administering to the individual an isolated EBA-175 antibody in an amount effective to inhibit *P. falciparum* invasion into the red blood cell.

The antibodies and peptides of the present invention may also be used to detect or quantify *P. falciparum* in a biological sample or specimen or culture media, or used in diagnostic methods and kits, as described below. Results from these tests can be used to predict or diagnose the occurrence or recurrence of *P. falciparum* mediated diseases such as malaria. Antibodies to the *P. falciparum* erythrocytic binding proteins may also be used in production facilities or laboratories to isolate additional quantities of the *P. falciparum* erythrocytic binding proteins, such as by affinity chromatography, or for the development of peptide agonists or antagonists.

P. falciparum related diseases such as malaria are prevented or treated by administering to a patient suffering from a *P. falciparum* related disease, a pharmaceutical composition containing substantially purified EBA-175 antibodies, EBA-175 peptides, EBA-175 polypeptide agonists or antagonists, or EBA-175 polypeptide antisera. Additional prevention and treatment methods include administration of EBA-175 antibodies, EBA-175

peptides, EBA-175 polypeptide antisera, or EBA-175 receptor agonists and antagonists linked to cytotoxic or anti-parasitic agents.

The antibodies specific for EBA-175 may be administered to a patient to passively immunize the patient against *P. falciparum* infection, thereby reducing *P. falciparum* related diseases such as malaria. The blocking peptides that specifically inhibit binding of *P. falciparum* to a red blood cell may also be administered to a patient to actively immunize the patient against *P. falciparum* infection, thereby reducing *P. falciparum* related diseases such as malaria. Administration of the EBA-175 antibodies or EBA-175 peptides may occur prior to any signs of *P. falciparum* infection. Such an administration would be important in individuals in areas where *P. falciparum* is endemic, or to individuals planning to travel to endemic areas. Administration of the EBA-175 antibodies and peptides may also occur after signs of *P. falciparum* infection have surfaced in order to interrupt the life cycle of the *Plasmodium* parasite and inhibit the spread of the organism.

In a preferred embodiment, a vaccine for passive or active immunization against malaria is packaged in a single dosage for immunization by parenteral (i.e., intramuscular, intradermal or subcutaneous) administration. The vaccine is most preferably injected intramuscularly into the deltoid muscle. The vaccine is preferably combined with a pharmaceutically acceptable carrier to facilitate administration. The carrier is usually water or a buffered saline, with or without a preservative. The vaccine may be lyophilized for re-suspension at the time of administration or in solution.

The carrier to which the antibody or peptides may be conjugated may also be a polymeric delayed release system. Synthetic polymers are particularly useful in the formulation of a vaccine to effect the controlled release of antibody. Microencapsulation of the antibody or peptide will also give a controlled release. A number of factors contribute to the selection of a particular polymer for microencapsulation. The reproducibility of polymer synthesis and the microencapsulation process, the cost of the microencapsulation materials and process, the toxicological profile, the requirements for variable release kinetics and the physicochemical compatibility of the polymer and the antigens are all factors that must be considered. Examples of useful polymers are polycarbonates, polyesters, polyurethanes, polyorthoesters polyamides, poly (d,l-lactide-co-glycolide) (PLGA) and other biodegradable polymers.

The preferred dose for human administration of the pharmaceutical composition or vaccine is from 0.01 mg/kg to 10 mg/kg. Based on this range, equivalent dosages for heavier body weights can be determined. The dose should be adjusted to suit the individual to whom the composition is administered and will vary with age, weight and metabolism of the individual. The vaccine may additionally contain stabilizers such as thimerosal (ethyl(2-mercaptobenzoate-S)mercury sodium salt) (Sigma Chemical Company, St. Louis, MO) or physiologically acceptable preservatives.

The antibodies of the present invention may also be used for the detection of *P. falciparum* peptides in biological samples or culture media. There are many techniques known in the art for detecting a component such as a polypeptide in a mixture and/or measuring its amount. Immunoassays, which employ antibodies that bind specifically to the polypeptide of interest, are one of the better known measurement techniques. Classical methods involve reacting a sample containing the polypeptide with a known excess amount of antibody specific for the polypeptide, separating bound from free antibody, and determining the amount of one or the other. Often the antibody is labeled with a reporter group to aid in the determination of the amount of bound analyte as described above. The reporter group or "label" is commonly a fluorescent or radioactive group or an enzyme.

An immunoassay is performed for the detection of *P. falciparum* in a sample as follows:

A sample is collected or obtained using methods well known to those skilled in the art. The sample containing the *P. falciparum* polypeptides to be detected may be obtained from an culture media or any biological source. Examples of biological sources include blood serum, blood plasma, urine, spinal fluid, fermentation fluid, lymph fluid, tissue culture fluid and ascites fluid. The sample may be diluted, purified, concentrated, filtered, dissolved, suspended or otherwise manipulated prior to immunoassay to optimize the immunoassay results.

To detect *P. falciparum* polypeptides, the sample is incubated with one or more of the *P. falciparum* erythrocyte binding protein antibodies of the present invention. The antibody may be labeled or conjugated to a solid phase bead or particle as also described herein. The labeled antibody is then detected using methods well known to those skilled in the art. The term "detecting" or "detected" as used herein means using known techniques for

detection of biologic molecules such as immunochemical or histological methods. Such methods include immunological techniques employing monoclonal or polyclonal antibodies to the peptides, such as enzyme linked immunosorbant assays, radioimmunoassay, chemiluminescent assays, or other types of assays involving antibodies known to those skilled in the art.

Current binding assay technology benefits from the diversity of detection systems developed that use enzyme-catalyzed chromogenic reactions, radionuclides, chemiluminescence, bioluminescence, fluorescence, fluorescence polarization and a variety of potentiometric and optical biosensor techniques.

Binding assays rely on the binding of analyte by analyte receptors to determine the concentrations of analyte in a sample. Analyte-receptor assays can be described as either competitive or non-competitive. Non-competitive assays generally utilize analyte receptors in substantial excess over the concentration of analyte to be determined in the assay. Sandwich assays are examples of non-competitive assays, that comprise one analyte receptor frequently bound to a solid phase and a second analyte receptor labeled to permit detection. The analyte first binds to the analyte receptor bound to a solid phase and the second labeled analyte receptor is then added to facilitate quantitation of the analyte. Bound analyte can easily be separated from unbound reagents, such as unbound labeled first analyte receptors, due to the use of an analyte receptor bound to a solid phase.

Competitive assays generally involve a sample suspected of containing analyte, an analyte-analogue conjugate, and the competition of these species for a limited number of binding sites provided by the analyte receptor. Competitive assays can be further described as being either homogeneous or heterogeneous. In homogeneous assays all of the reactants participating in the competition are mixed together and the quantity of analyte is determined by its effect on the extent of binding between analyte receptor and analyte-conjugate or analyte analogue-conjugate. The signal observed is modulated by the extent of this binding and can be related to the amount of analyte in the sample. U.S. Patent No. 3,817,837 describes such a homogeneous, competitive assay in which the analyte analogue conjugate is a analyte analogue-enzyme conjugate and the analyte receptor, in this case an antibody, is capable of binding to either the analyte or the analyte analogue. The binding of the antibody to the analyte analogue-enzyme conjugate decreases the activity of the enzyme relative to the activity observed when the enzyme is in the unbound state. Due to competition

between unbound analyte and analyte analogue-enzyme conjugate for analyte-receptor binding sites, as the analyte concentration increases the amount of unbound analyte analogue-enzyme conjugate increases and thereby increases the observed signal. The product of the enzyme reaction may then be measured kinetically using a spectrophotometer.

Heterogeneous, competitive assays require a separation of analyte analogue conjugate bound to analyte receptor from the free analyte analogue conjugate and measurements of either the bound or the free fractions. Separation of the bound from the free may be accomplished by removal of the analyte receptor and anything bound to it from the free analyte analogue conjugate by immobilization of the analyte receptor on a solid phase or precipitation. The amount of the analyte analogue conjugate in the bound or the free fraction can then be determined and related to the concentration of the analyte in the sample. Normally the bound fraction is in a convenient form, for example, on a solid phase, so that it can be washed, if necessary, to remove remaining unbound analyte analogue conjugate and the measurement of the bound analyte analogue conjugate or related products is facilitated. The free fraction is normally in a liquid form that is generally inconvenient for measurements. If multiple analytes are being determined in a single assay, the determination of the free fraction of analyte analogue conjugate for each analyte is made impossible if all are mixed in a single liquid unless the responses of the individual analyte analogue conjugates can be distinguished in some manner. However, detecting the free fraction of analyte analogue conjugate in assays that are visually interpreted is a distinct advantage because the density of the color developed in such assays is generally proportional to the analyte concentration over much of the range of analyte concentration.

In a preferred embodiment, the method for detecting and characterizing *P. falciparum* polypeptides comprises taking a sample from a protein production lot. A determination of the presence of the immunodominant polypeptides can then be made using assay techniques that are well known to those skilled in the art and include methods such as Western blot analysis, radioimmunoasssay and ELISA assays.

In a second preferred embodiment, the method for detecting *P. falciparum* polypeptides comprises taking biological samples, such as fluids and tissues, from a mammal for the diagnosis or prognosis of malaria. The sample is preferably obtained from the blood, cerebrospinal fluid, urine or tissues of a

mammal, preferably a human or simian. A determination of the presence of the immunodominant polypeptides can then be made using assay techniques that are well known to those skilled in the art and include methods such as Western blot analysis, radioimmunoasssay and ELISA assays.

A kit for detecting the presence and quantity of *P. falciparum* peptides is also provided. The kit can be in any configuration well known to those of ordinary skill in the art and is useful performing one or more of the methods described herein for the detection of *P. falciparum* in biological samples or for the detection or monitoring of *P. falciparum* infection in a patient or carrier. The kits are convenient in that they supply many if not all of the essential reagents for conducting an assay for the detection of *P. falciparum* in a biological sample. The reagents may be premeasured and contained in a stable form in vessels or on a solid phase in or on which the assay may be performed, thereby minimizing the number of manipulations carried out by the individual conducting the assay. In addition, the assay may be performed simultaneously with a standard that is included with the kit, such as a predetermined amount of antigen or antibody, so that the results of the test can be validated or measured.

The kit preferably contains one or more *P. falciparum* erythrocyte binding protein antibodies that can be used for the detection of *P. falciparum* binding proteins in a sample. The kit can additionally contain the appropriate reagents for binding or hybridizing the antibodies to their respective *P. falciparum* binding molecules or ligands in the sample as described herein and reagents that aid in detecting the bound peptides. The kit may additionally contain equipment for safely obtaining the sample, a vessel for containing the reagents, a timing means, a buffer for diluting the sample, and a colorimeter, reflectometer, or standard against which a color change may be measured.

In a preferred embodiment, the reagents, including the antibody, are lyophilized, most preferably in a single vessel. Addition of aqueous sample to the vessel results in solubilization of the lyophilized reagents, causing them to react. Most preferably, the reagents are sequentially lyophilized in a single container, in accordance with methods well known to those skilled in the art that minimize reaction by the reagents prior to addition of the sample.

The assay kit includes but is not limited to reagents to be employed in the following techniques; competitive and non-competitive assays, radioimmunoassay, bioluminescence and chemiluminescence assays, fluorometric assays, sandwich assays, immunoradiometric assays, dot blots,

enzyme linked assays including immunoblots and ELISAs, and immunocytochemistry. Materials used in conjunction with these techniques include, but are not limited to, microtiter plates, antibody coated strips or dipsticks for rapid monitoring of urine or blood. For each kit, the range, sensitivity, precision, reliability, specificity and reproducibility of the assay are established. Intraassay and interassay variation is established at 20%, 50% and 80% points on the standard curves of displacement or activity.

In a further preferred embodiment, the assay kit uses immunoblot techniques and provides instructions, *P. falciparum* polypeptides, and *P. falciparum* erythrocyte binding protein antibodies conjugated to a detectable molecule. The kit is useful for the measurement of *P. falciparum* in biological fluids and tissue extracts of animals and humans with and without malaria, as well as in culture media.

This invention is further illustrated by the following examples, which are not to be construed in any way as imposing limitations upon the scope thereof. On the contrary, it is to be clearly understood that resort may be had to various other embodiments, modifications, and equivalents thereof which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the spirit of the present invention and/or the scope of the appended claims.

Example 1

Production of Monoclonal Antibodies that Bind EBA-175

Mouse monoclonal antibodies were produced by the protocol as described in Harlow and Lane (1988) *Antibodies, A Laboratory Manual*, Cold Spring Harbor Publications, New York. Briefly, Balb/c mice were immunized with a purified recombinant baculovirus EBA-175 region II protein as an immunogen. Immune mice were used as splenocyte donors for fusion with a syngeneic mouse fusion partner, Sp2/0-Ag14. Hybridomas were selected based on the following criteria:

1. EBA -175 subcellular apical localization patterns on parasitized erythrocytes as determined by immunofluorescence assays;
2. ELISA positivity against a recombinant baculovirus region II protein; and

3. Neutralization of native EBA-175 binding to human erythrocytes.

Hybridomas that were selected were cloned twice by limiting dilution and maintained at -180° C.

Example 2

Characterization of Monoclonal Antibodies that Bind EBA-175

Four IgG1 monoclonal antibodies labeled R215, R216, R217 and R256 were analyzed for the ability to block the binding of [^{35}S]methionine labeled EBA-175 to erythrocytes. Monoclonal antibodies R215, R217 and R256 inhibited greater than 90% binding, while R216 inhibited approximately 30% binding. (Data not shown). An RII EBA-175 region peptide expressed in baculovirus and RII, RII/F1 and RII/F2 EBA-175 region peptides expressed in yeast were separated by SDS-PAGE and probed with labeled monoclonal antibodies R216 and R217. Both the R216 and R217 antibodies did not recognize the RII/F1 region, but specifically recognized the RII/F2 region of EBA-175. (Data not shown). The most effective neutralizing antibodies, R215, R217 and R256, recognize a secondary structural epitope within the 5' cysteine rich region designated RII/F2. (Data not shown). In contrast, monoclonal antibody R216 recognizes a linear epitope within the 5' cysteine rich region designated RII/F2. (Data not shown).

Additionally, monoclonal antibodies R215, R216, R217 and R256 were tested for their ability to neutralize merozoite invasion *in vitro*. As compared to control monoclonal antibodies, R215, R217 and R256 blocked 82%, 83% and 76%, respectively, of merozoite invasion in the homologous FVO strain. Additionally, R215, R217 and R256, blocked 35%, 39% and 37%, respectively, of merozoite invasion in the heterologous 3D7 strain. (See Figure 3).

Example 3

Amino Acid Sequence of EBA-175 Peptides

Following panning against a constrained heptapeptide M13 phage display library using EBA-175 antibody R256, the amino acid sequence of several peptides was determined. Six of eleven peptides contained the sequence of PEP R256-2, while one of the peptides contained the sequence of PEP R256-1. (See Figure 2).

Modifications and variations of the present method will be obvious to those skilled in the art from the foregoing detailed description. Such modifications and variations are intended to come within the scope of the appended claims.

References

Camus, D. and T. J. Hadley. "A *P. falciparum* antigen that binds to host erythrocytes and merozoites." *Science.* 230(4725): 553-6 (1985).

Chitnis, C. E. and L. H. Miller. "Identification of the erythrocyte binding domains of *P. vivax* and *P. knowlesi* proteins involved in erythrocyte invasion." *J Exp Med* 180(2): 497-506 (1994).

Hadley, T. J. "Invasion of erythrocytes by malaria parasites: a cellular and molecular overview." *Annu Rev Microbiol* 40: 451-77 (1986).

Sim, B. K., P. A. Orlandi, et al. "Primary structure of the 175K *P. falciparum* erythrocyte binding antigen and identification of a peptide which elicits antibodies that inhibit malaria merozoite invasion." *J Cell Biol* 111(5 Pt 1): 1877-84 (1990).

Fang, X., Kaslow, D. C., Adams, J. H., Miller, L. H. "Cloning of the *P. vivax* Duffy receptor." *Mol. Biochem. Parasitol.* 44: 125-132 (1991).

Adams, J. H., Hudson, D. E., Torii, M., Ward, G. E., Wellems, T. E., Aikawa, M., Miller, L. H. "The Duffy receptor family of *P. knowlesi* is located within the micronemes of invasive malaria merozoites." *Cell.* 63: 141-153 (1990).

Adams, J.H., Sim, B.K.L., Dolan, S.A., Fang, X., Kaslow, D.C., Miller, L.H. "A family of erythrocyte binding proteins of malaria parasites." *Proc. Natl. Acad. Sci.* 89: 7085-7089 (1992).

Orlandi, P. A., Klotz, F. W. and Haynes, J. D. "A malaria invasion receptor, the 175-kilodalton erythrocyte binding antigen of *P. falciparum* recognizes the

terminal neu5Ac(α2-3)gal-sequences of glycophorin A." *J. Cell Biol.* 116: 901-909 (1992).

Sim, B.K.L., Chitnis, C.E., Wasniowska, K., Hadley, T.J., Miller, L.H. "Receptor and ligand domains for invasion of erythrocytes by *P. falciparum* ." *Science.* 264:1941-1944 (1994).

Claims

We claim:

1. An isolated antibody, wherein the antibody binds a 5' cysteine rich region of an EBA-175 protein from a *Plasmodium* species.

2. The isolated antibody of Claim 1, wherein the 5' cysteine rich region is a region II.

3. The isolated antibody of Claim 1, wherein the 5' cysteine rich region is a region II/F2.

4. The isolated antibody of Claim 1, wherein the region consists of an approximately 10 amino acid sequence.

5. The isolated antibody of Claim 4, wherein the amino acid sequence is shown in SEQ ID NO:1.

6. The isolated antibody of Claim 1, wherein the *P.* species is *Plasmodium falciparum*.

7. The isolated antibody of Claim 1, wherein the antibody inhibits binding of an EBA-175 protein to a red blood cell.

8. The isolated antibody of Claim 1, wherein the antibody inhibits *Plasmodium falciparum* invasion into a red blood cell.

9. The isolated antibody of Claim 8, wherein the antibody inhibits *Plasmodium falciparum* invasion of a red blood cell *in vitro*.

10. The isolated antibody of Claim 8, wherein the antibody inhibits *Plasmodium falciparum* invasion of a red blood cell *in vivo*.

11. The isolated antibody of Claim 1, wherein the antibody is monoclonal.

12. A hybridoma producing the monoclonal antibody of Claim 11.

13. A method of treating a *Plasmodium* species related disease comprising, administering to an individual the isolated antibody of Claim 1 in an amount effective to treat the Plasmodium species related disease.

14. The method of Claim 13, wherein the *Plasmodium* species is *Plasmodium falciparum*.

15. The method of Claim 14, wherein the *Plasmodium falciparum* related disease is malaria.

16. A method of inhibiting *Plasmodium falciparum* invasion into a red blood cell of an individual comprising, administering to the individual the isolated antibody of Claim 1 in an amount effective to inhibit *Plasmodium falciparum* invasion into the red blood cell.

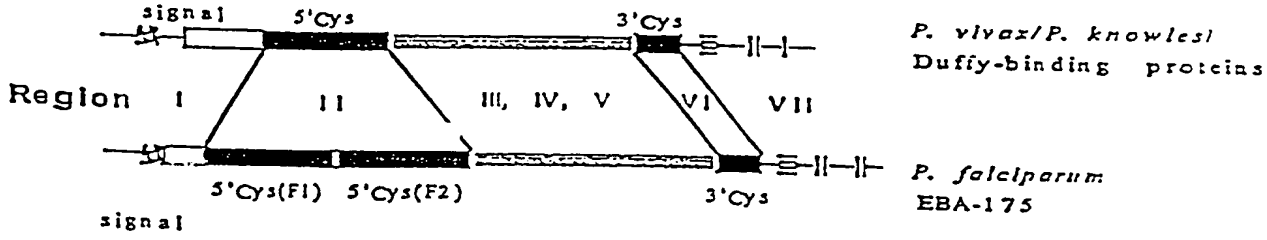

signal
5'Cys
3'Cys
P. vivax/P. knowlesi
Duffy-binding proteins
Region I II III, IV, V VI VII
5'Cys(F1) 5'Cys(F2)
3'Cys
P. falciparum
EBA-175
signal

FIG. 1

		Frequency
	344 353	
EBA-175 RII	C V P P R R Q E L C	
	| | • |	
PEP R256-1	C N M V P M S R C	1/11
PEP R256-2	C W S I N P R W C	6/11

FIG. 2

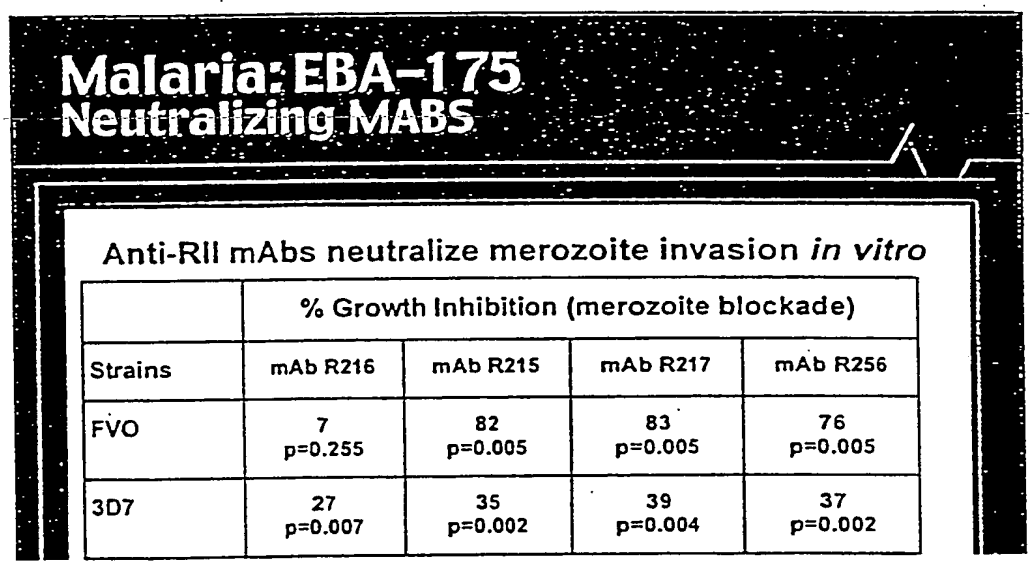

Malaria: EBA-175
Neutralizing MABS
Anti-RII mAbs neutralize merozoite invasion in vitro
% Growth Inhibition (merozoite blockade)
Strains
mAb R216
mAb R215
mAb R217
mAb R256
FVO
7
p=0.255
82
p=0.005
83
p=0.005
76
p=0.005
3D7
27
p=0.007
35
p=0.002
39
p=0.004
37
p=0.002

FIG. 3